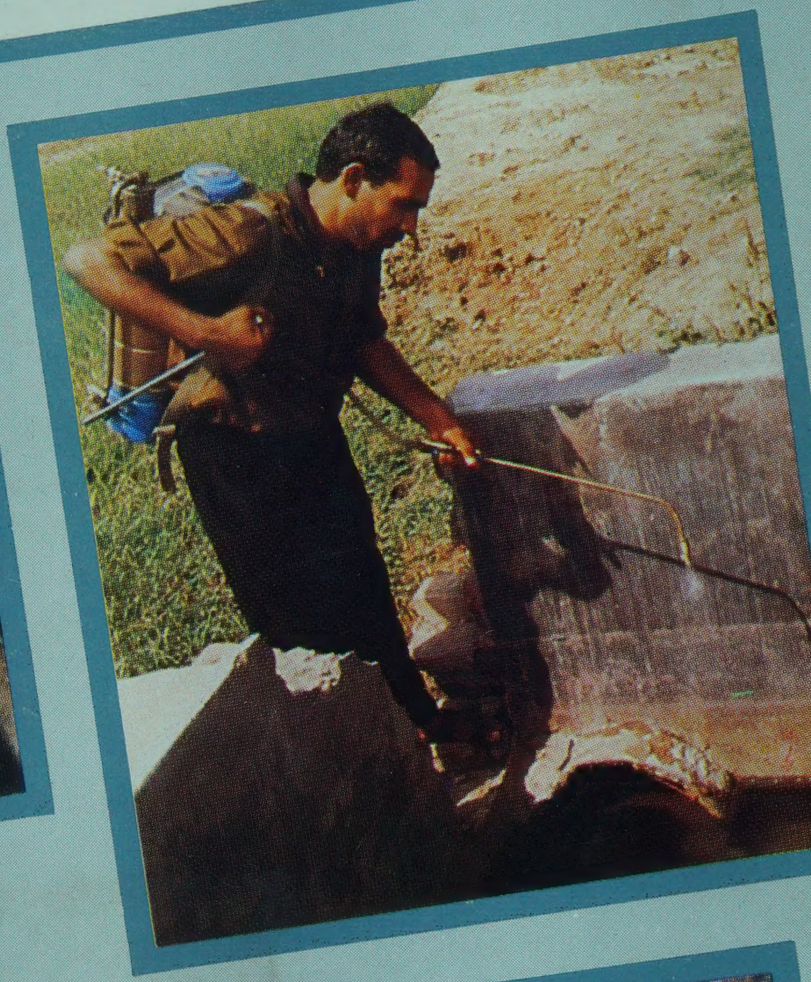
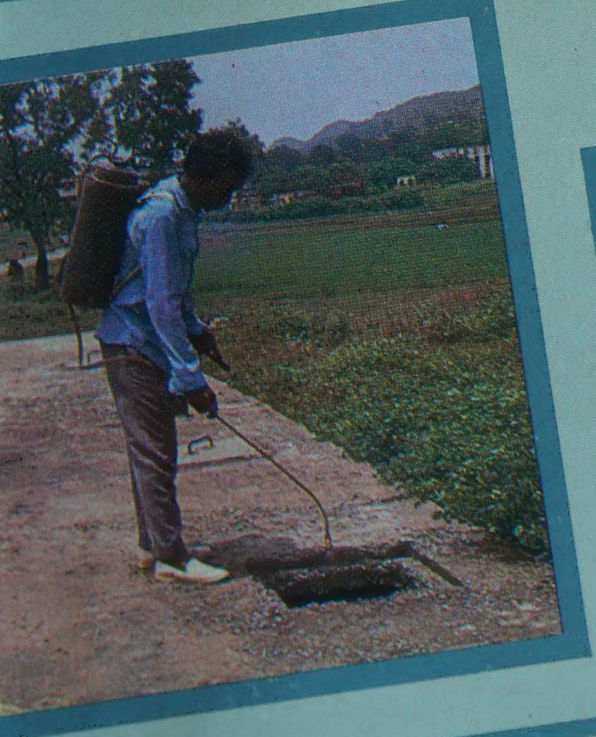
BIOLARVICIDES

(*Bacillus thuringiensis var. israelensis*; Serotype H-14)





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BIOLARVICIDES

Bacillus thuringiensis var. *israelensis* serotype H-14 (Bti)

Bacillus thuringiensis var. *israelensis* serotype H-14 (Bti) is the most widely used microbial agent that acts specifically on the larvae of mosquitoes and blackflies. Commercially Bti is available in various formulations which are often referred as Biolarvicides. The information given in the brochure is based on a wettable powder formulation of Bti namely "BACTICIDE".

WHY BIOLARVICIDES ?

Biolarvicide has the following advantages over chemical larvicides.

Advantages	Biolarvicides (Bti)	Chemical larvicides *
Safety to environment	Yes	No
Safety to human/animals	Yes	No
Kills mosquito predators and parasites	No	Yes
Prone to theft/misuse	No	Yes
Alternate use of larvicides	No	Yes
Application cycle	Fortnightly	Weekly
Acceptability by the communities	High	Indifferent
Environment-friendly	Yes	No
Storage and handling	Easy	?

* Fenthion and abate are commonly used in India.

The Organism

Bacillus thuringiensis var. *israelensis* serotype H-14 (Bti) is aerobic, gram positive, rod shaped, spore forming bacteria. Though *Bacillus thuringiensis* is known to have insecticidal properties since early 20th century, serotype H-14, based on identification through H-antigens, namely the flagellar proteins, was isolated in 1976. During the maturation phase of growth bacterial cells produce bipyramidal protein crystal close to the spores, therefore called "parasporal crystal".

The protein-crystal is a protoxin also called delta-endotoxin, which on activation in the insect midgut releases fractions toxic to mosquitoes and blackflies.

Insecticide Potency

The product being biological, its active principle is usually determined by biological assay directly related to the efficacy on target insects. This is a more appropriate method to estimate the sum effect of spore-crystal complex, Bioassay of Bti is usually carried out against IV instar larvae of *Aedes aegypti* as Ld_{50} value in mg/L of product. Against *Ae. aegypti* its potency is around 1000 ITU/mg compared to IPS 82 reference standard powder.

Alternatively, delta-endotoxin content can be measured as per cent weight by weight, spectrophotometrically at 280 nm, after extraction and precipitation in comparison to known standard. Normally 5-8% endotoxin is present in the standard product. Both the assay methods are used for quality control of the commercial product.

Biological Properties

Bacticide, the wettable powder formulation of Bti is active only against the larval stages. The pupae and adult are not susceptible. The II, III and early IV instar larvae are most

susceptible. Late IV instar shows little effect due to cessation of feeding by larvae and no uptake of spore-crystal complex.

Quick knock out activity within few hours of application is observed obtaining a total kill within 24 hours. Cent per cent kill of some species of mosquitoes is exhibited within 4-8 hrs of application.

The biolarvicide persists in nature from 7-28 days depending upon the rate of application and other environmental factors.

Being a target-specific toxin, the biolarvicide has no action on other hydrobionts, benefical insects and warm blooded animals (mammals).

Physico-Chemical Properties of Bacticide

Physical state	Homogeneous dry powder
Appearance	Light grey to grey brown colour
Moisture content	Not more than 7%
Particle size	> 90 μ = Not more than 5% >500 μ = Not more than 0.3%
pH	6.0 - 6.6
Viable spore count	Around 30 billion spores/gm
Suspensibility in water	Excellent
Shelf Life	2 years when stored in original sealed packaging, temperature < 40°C

Mode of Action

Bti acts as stomach poison against mosquito larvae through its parasporal crystal delta-endotoxin. Mosquito larvae ingest these toxins alongwith other organic matter. In the mid gut of larvae, toxins are activated by alkaline pH and proteinases. The activated toxin pass through the peritrophic membrane and binds with the specific receptor in midgut epithelial cells and makes the cell wall porous, leading to rapid changes in the permeability, followed by disintegration of midgut epithelium and, thereby the gut wall.

The disintegration of gut wall results in immediate cessation of feeding leading to starvation. It is followed by gut paralysis and leakage of gut contents into haemolymph and body cavity of the larvae. Lesions in the wall lead to paralysis and death of larva.

Concurrently, the spores germinate and bacterial cells that reproduce and multiply in the larval gut and body causes septicemia.

Table 1. Comparative toxicity of Bacticide (Bti) against III instar mosquito larvae

Mosquito species	Concentrations (mg/L) at 27± 1°C	
	LC_{50}	LC_{90}
An. culicifacies	0.32	1.1
An. stephensi	0.16	0.92
Cx. quinquefasciatus	0.062	0.27
Aedes aegypti	0.034	0.098

Effect of Temperature and pH

Various environmental factors such as temperature and water pH influence the efficacy of biolarvicide. Low temperature reduces the efficacy of biolarvicides.

Table 2. Effect of temperature on the larvicidal activity of Bacticide

Mosquito species	LC_{50} values (mg/L) with 95% confidence limits at two different temperature	
	21°C	31°C
An.culicifacies	0.8(0.635-1.01)	0.17(0.13-0.22)
An.stephensi	0.25(0.192-0.32)	0.076(0.061-0.094)
Cx.quinquefasciatus	0.19(0.15-0.23)	0.02(0.017-0.027)
Ae.aegypti	0.07(0.056-0.088)	0.021(0.018-0.25)

At pH 10 or more effectiveness of biolarvicides is greatly reduced

Table 3. Effect of water pH on the larvicidal activity of Bacticide

Mosquito Species	Per cent mortality				
	Conc. mg/L	3.5 pH	7.5 pH	9.5 pH	10.5 pH
An. stephensi	0.5	78	90	94	8
Cx. quinquefasciatus	0.25	100	98	88	12

Environmental Safety

The biolarvicide was found safe to various non-target species.

Table 4. Toxicity of Bacticide to certain non-target species

Species	LC_{50} (mg/L)
Gambusia affinis	>1000
Poecilia reticulata	>1000
Frog tadpoles	>1000
Notonectid bugs	
Enithares indica	>100
Anisopes sardae	>100
Mesocyclops (copepods)	>2000

Toxicity to Mammals: Various studies conducted to asses the safety of Bti formulation against mammals have shown following results.

Table 5. Toxicological studies on mammals

Route	Animal	Effect
Oral	Mice	No toxic effect Non-pathogenic
	Rabbit	No toxic effect Non-pathogenic

Contd.....

Table 5 (*Contd.....*)

Route	Animal	Effect
Intraperitoneal	Mice	No toxic effect Non-pathogenic
	Rabbit	No toxic effect Non-pathogenic
Mucous membrane	Mice	No irritation
	Rabbit	No irritation
Skin*	Mice	No irritation No sensitization
	Guinea pig	No irritation No sensitization
Inhalation*	Guinea pig	No toxic effect No sensitization
Oral subacute	Mice	No toxicity, Non-pathogenic
	Rat	No impact on post-natal development

* Bti formulation may cause allergic symptoms in some individuals.

Dosage: Bacticide (Bti) is to be applied at a dose of 0.5 gm/sq m (5 kg/hectare) against larvae of *Aedes, Culex* and *Anopheles* species.

Application: The wettable powder formulation of Bti can be applied with any larvicide application equipment prefer-

ably a knapsack sprayer. Initially a thick paste of the powder formulation is made with small quantity of water which is further diluted in 10 litre water to make the final suspension just before spraying. This is poured into the tank of sprayer through a fine mesh. The suspension should be used on the same day and every time fresh suspension should be made.

Monitoring: The impact of biolarvicide spraying is monitored by estimation of larval density in different habitats and adult mosquito density. The duration of the impact of biolarvicide is monitored by determining the per cent reduction in the density of III and IV instar larvae in the index breeding places. If III and IV instar larvae are found in index breeding places, the positive breeding habitats are to be treated with recommended dose of biolarvicides.

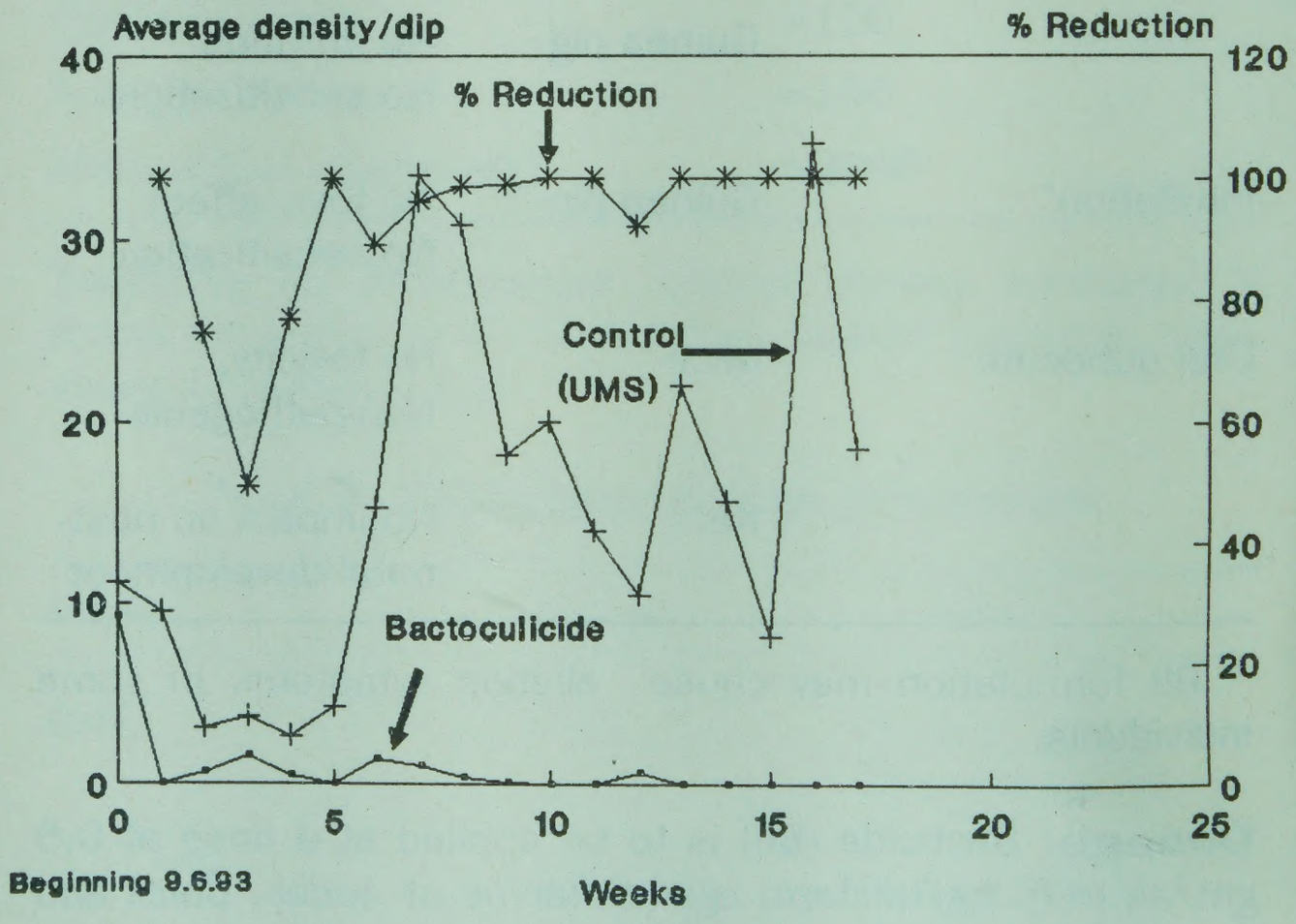

Fig. 1: Impact of spraying biolarvicide @ 0.5 gm/sq m on mosquito breeding in drains

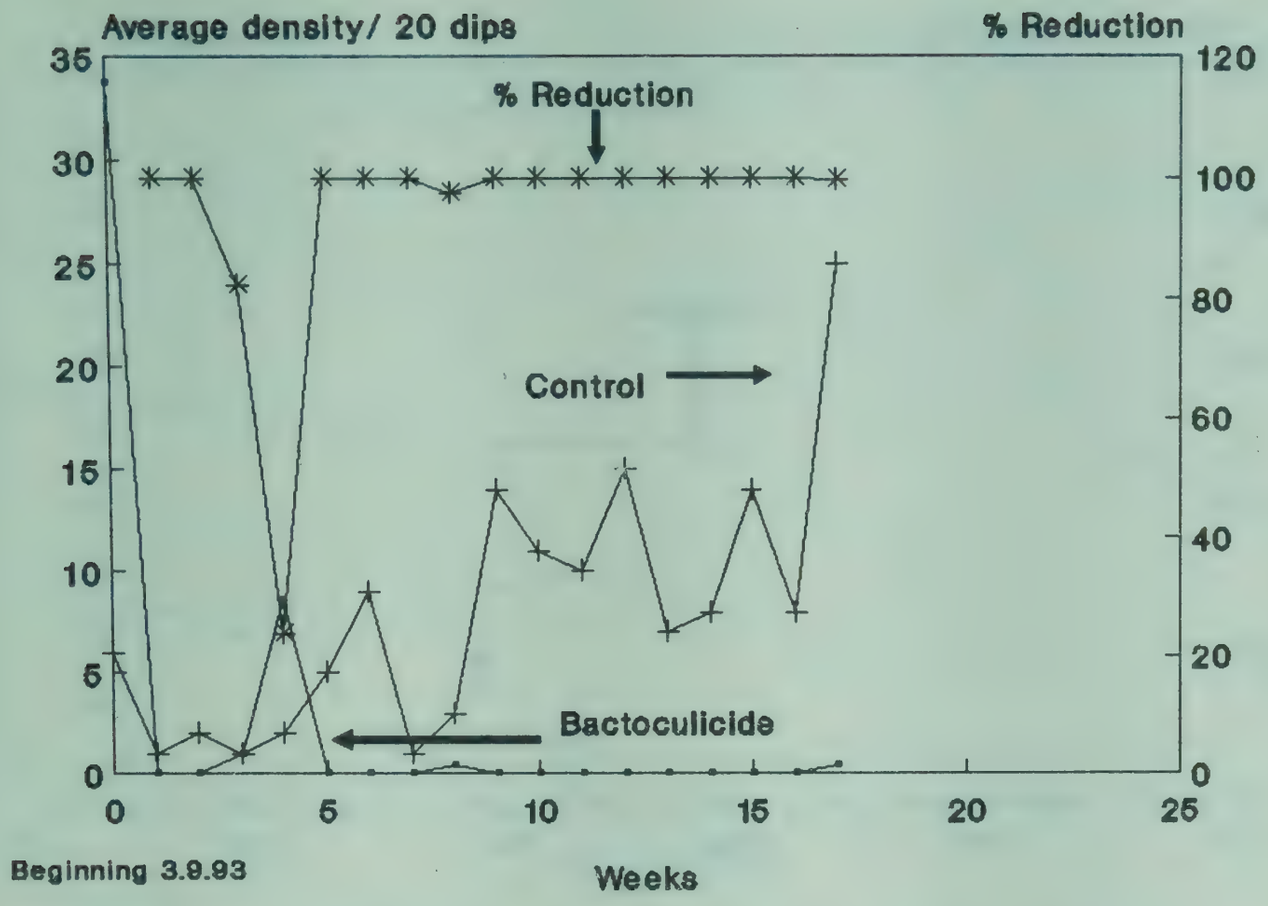

Fig. 2: Impact of spraying biolarvicide @ 0.5 gm/sq m on mosquito breeding in rice fields

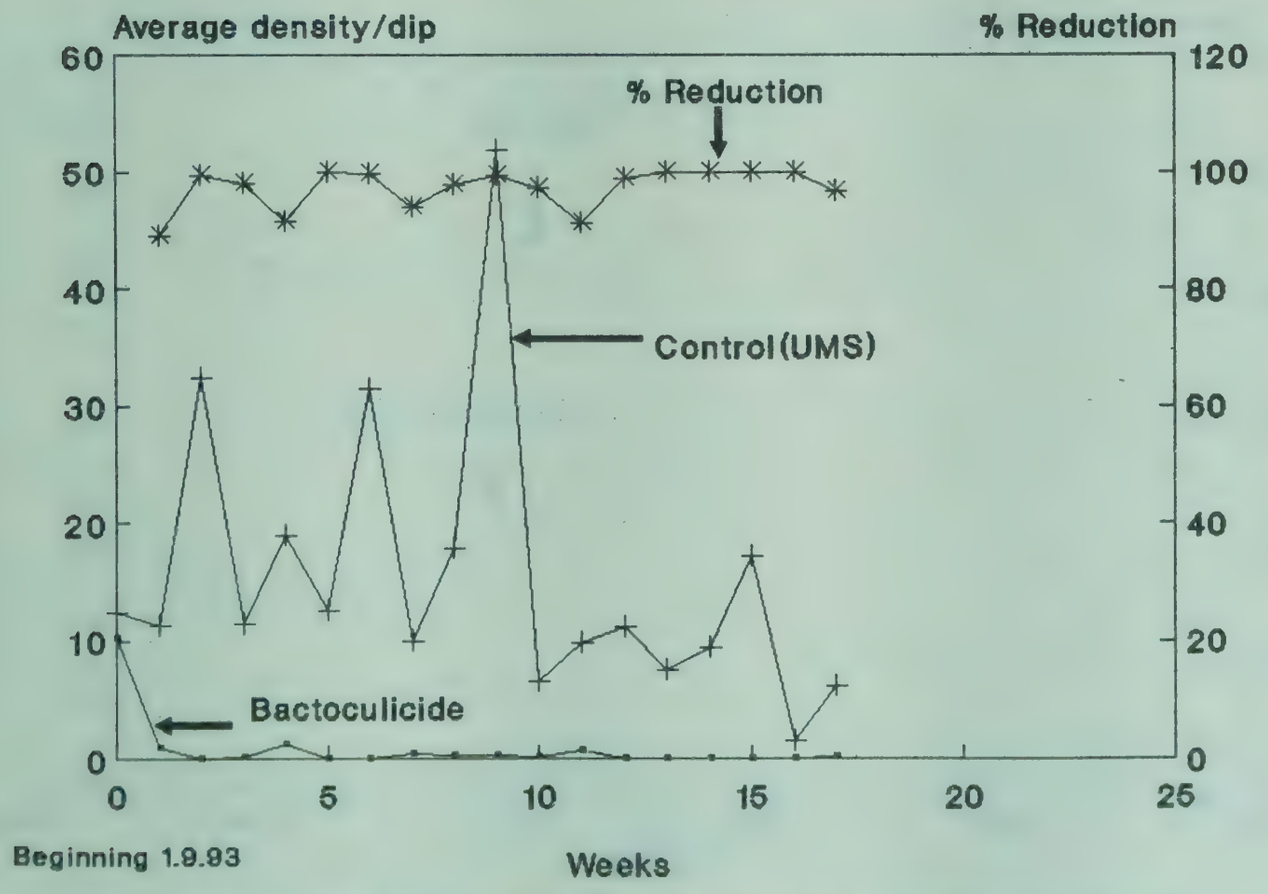

Fig. 3: Impact of spraying biolarvicide @ 0.5 gm/sq m on mosquito breeding in waste water pools

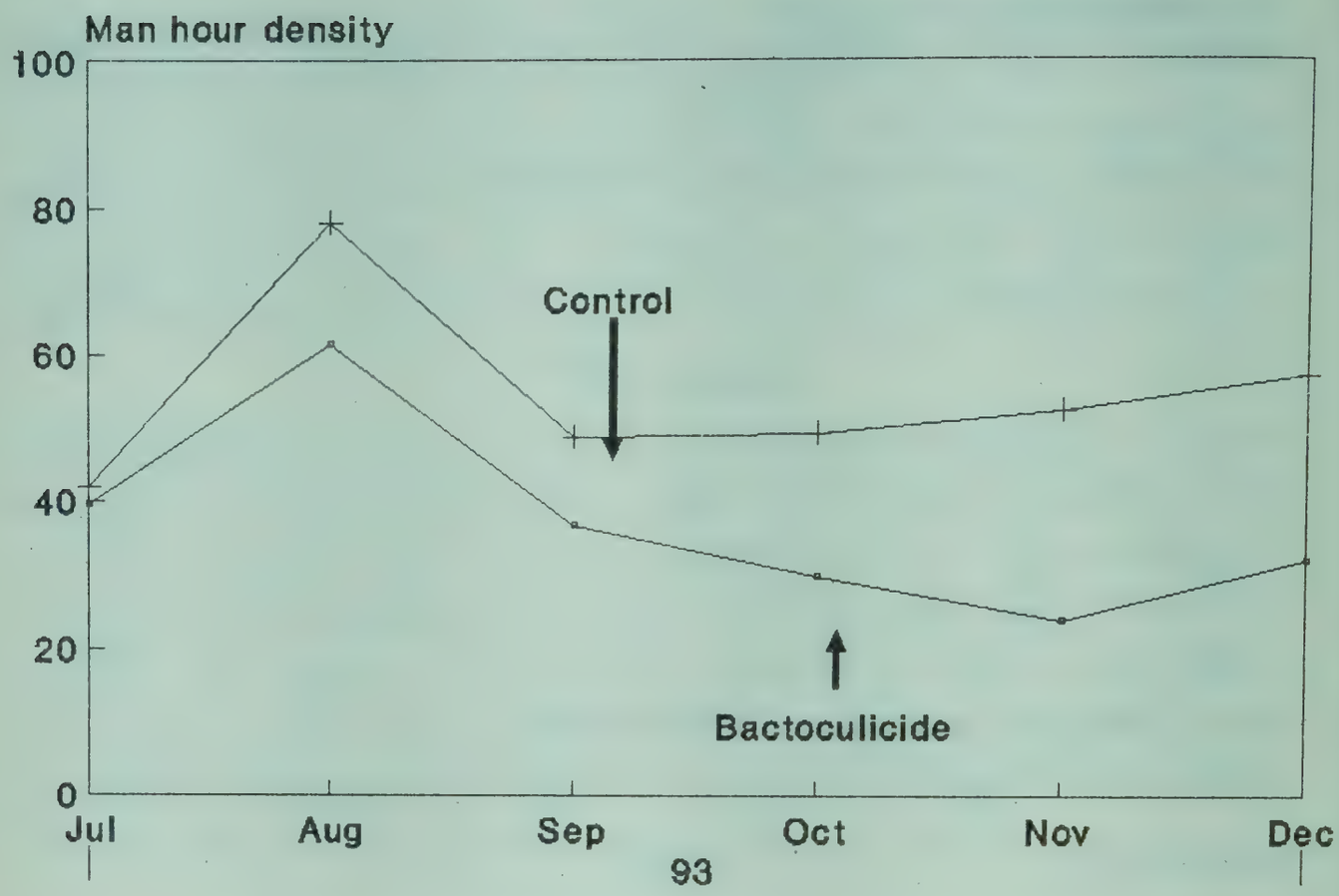

Fig. 4: Impact of spraying biolarvicide on the indoor density of total mosquitoes

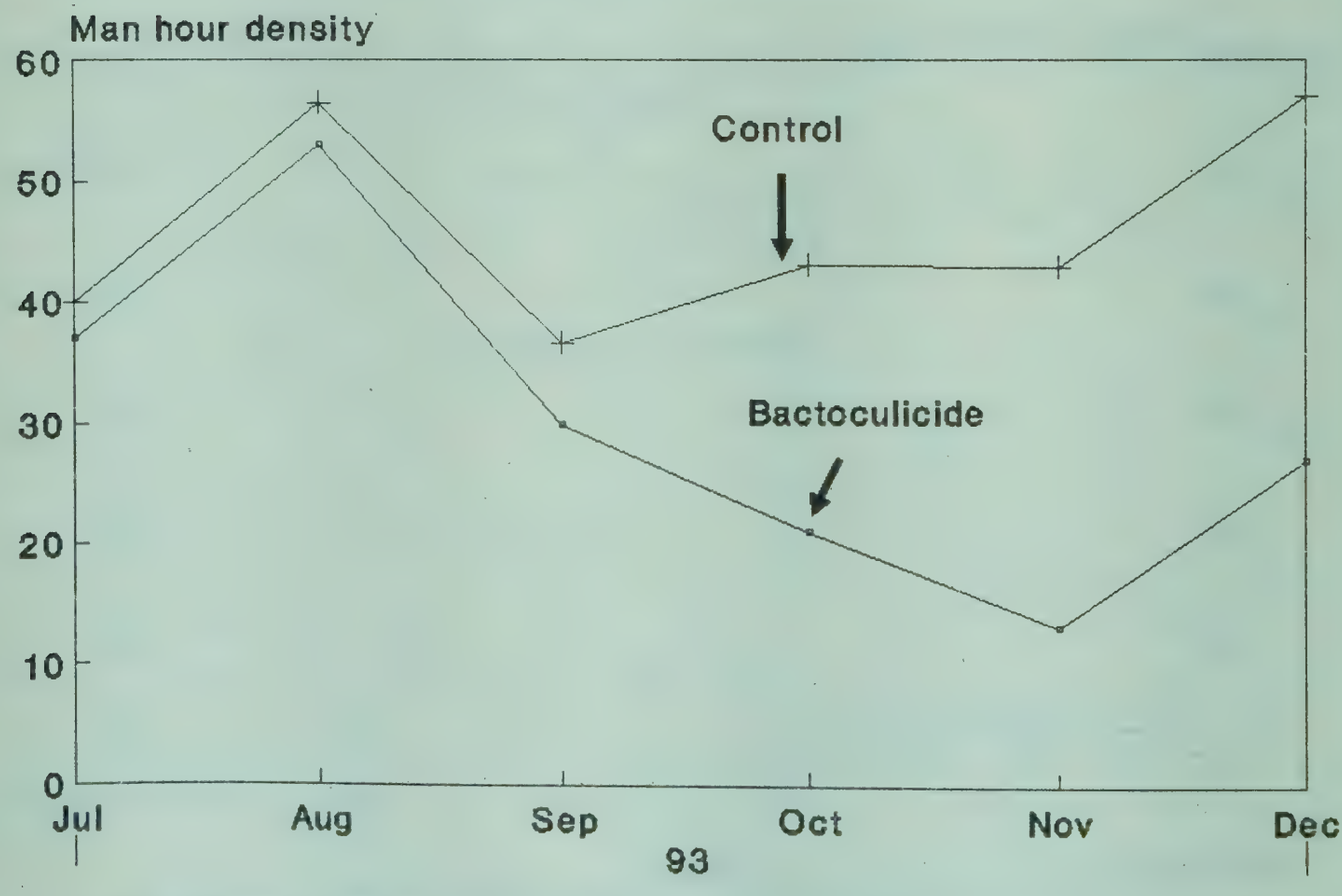

Fig. 5: Impact of spraying biolarvicide on the indoor density of *Cx. quinquefasciatus*

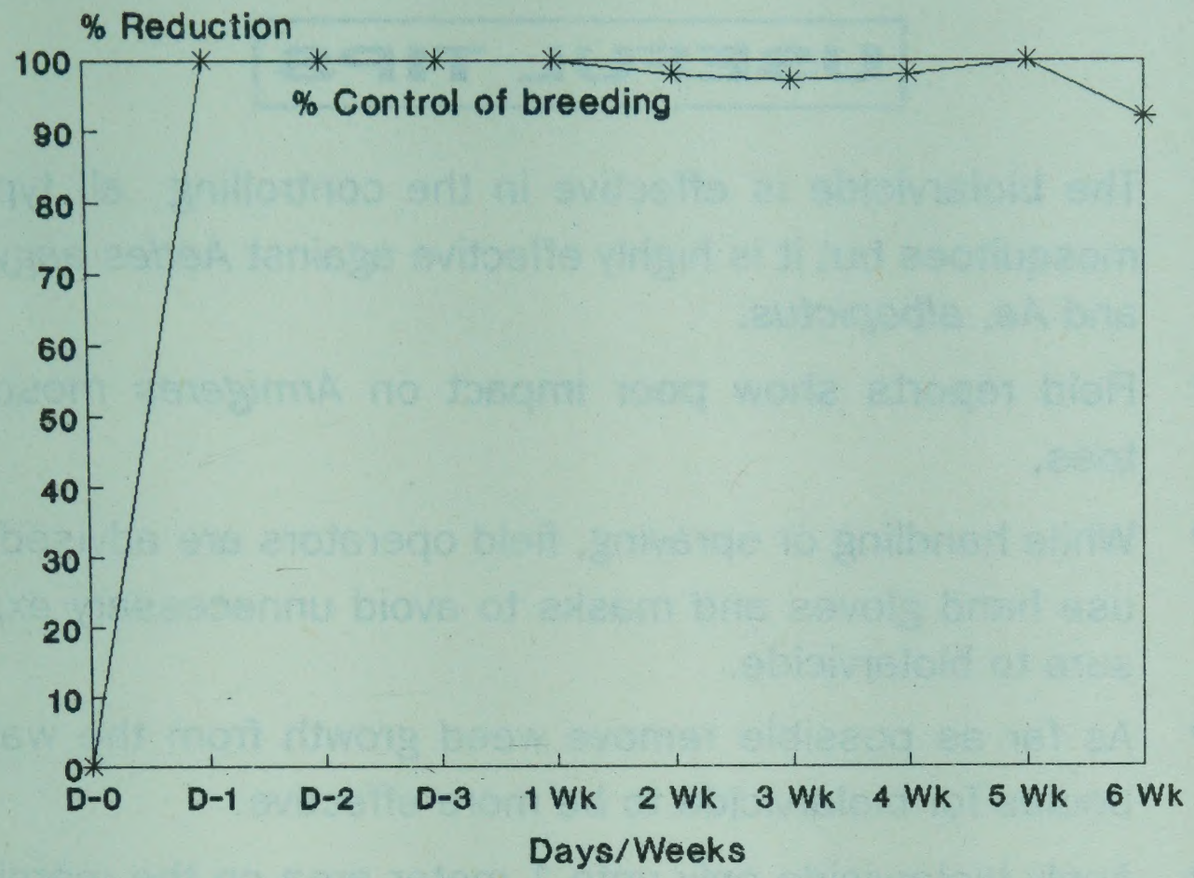

Fig. 6: Impact of biolarvicide spraying on *Ae. aegypti* and *Ae. albopictus* in industrial scraps

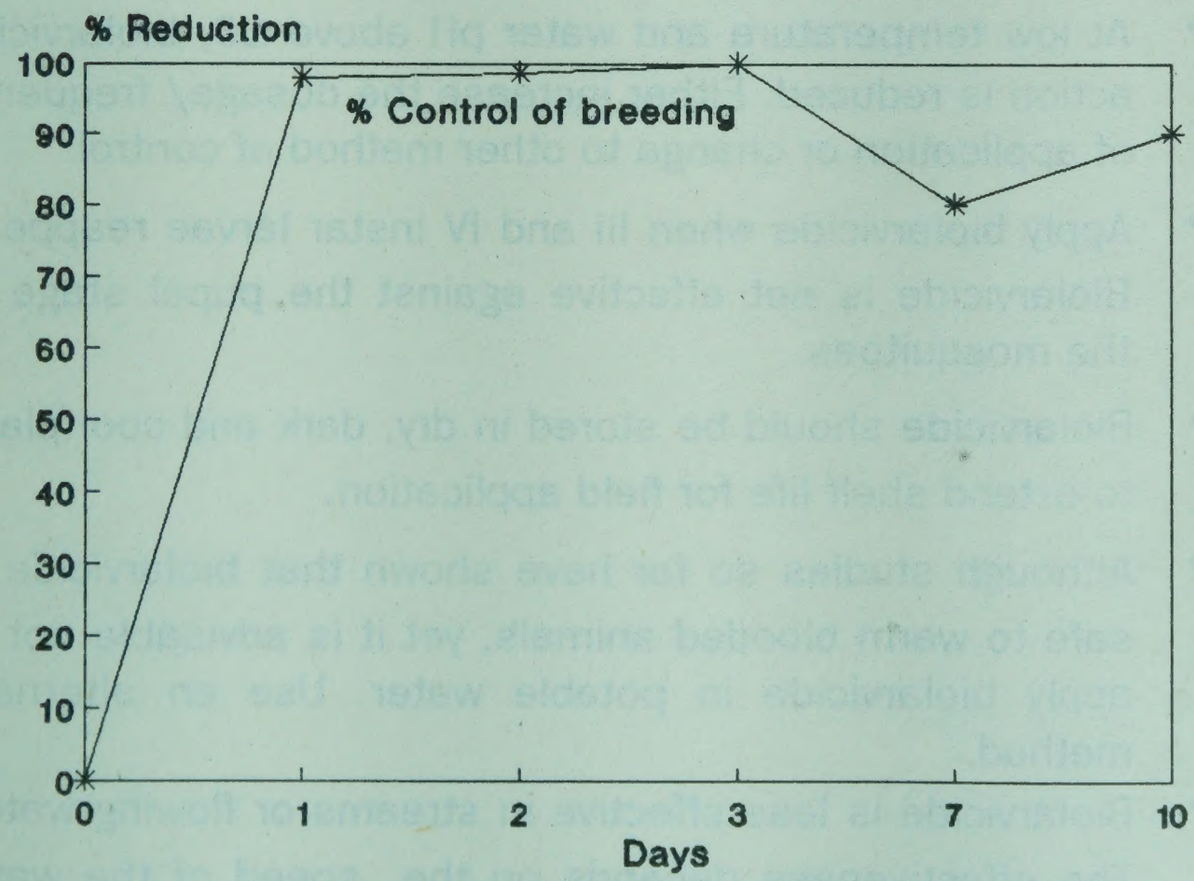

Fig. 7: Impact of biolarvicide spraying on anophelines in fresh water drains

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USEFUL TIPS

- The biolarvicide is effective in the controlling all types mosquitoes but it is highly effective against *Aedes aegypti* and *Ae. albopictus.*
- Field reports show poor impact on *Armigeres* mosquitoes.
- While handling or spraying, field operators are advised to use hand gloves and masks to avoid unnecessary exposure to biolarvicide.
- As far as possible remove weed growth from the water bodies for biolarvicide to be more effective.
- Apply biolarvicide only upto 1 meter area on the margins of the water body unless required due to weed growth inside the water bodies.
- At low temperature and water pH above 10, biolarvicide action is reduced. Either increase the dosage/ frequency of application or change to other method of control.
- Apply biolarvicide when III and IV instar larvae reappear. Biolarvicide is not effective against the pupal stage of the mosquitoes.
- Biolarvicide should be stored in dry, dark and cool place to extend shelf life for field application.
- Although studies so far have shown that biolarvicide is safe to warm blooded animals, yet it is advisable not to apply biolarvicide in potable water. Use an alternate method.
- Biolarvicide is less effective in streams or flowing water. The effectiveness depends on the speed of the water current.

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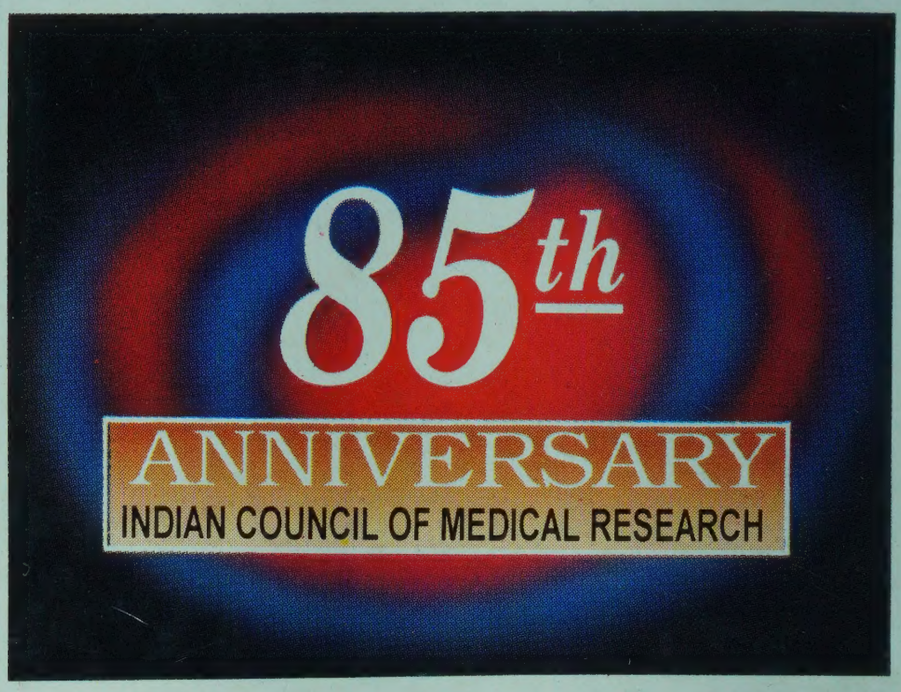

MALARIA RESEARCH CENTRE

The Malaria Research Centre (MRC) was established in the year 1977. Its primary task was to find short-term as well as long-term solutions to the problem of malaria through basic, applied and field operational research. The Centre is currently doing work in the areas of vector biology and control, genetics, cellular and molecular biology, parasitology, epidemiology, pharmacology and biochemistry, that is related to malariology and the development of malaria control strategies. A network of field laboratories in endemic areas provides the testing ground for new technologies and innovative approaches, and helps in the transfer of technology through training, field demonstrations and mass awareness programmes involving various media. The Centre also provides young scientists the opportunity to participate in advanced research through a fellowship programme. Close links in the form of scientific collaborations are maintained with WHO, NMEP and also leading national laboratories. Research findings of the Centre are published in reputed journals. Apart from this, the Centre publishes several books, monographs, proceedings including the Indian Journal of Malariology.